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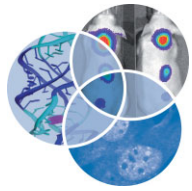

Viruses and the cellular RNA decay machinery

Marta Maria Gaglia and Britt A. Glaunsinger*

The ability to control cellular and viral gene expression, either globally or selectively, is central to a successful viral infection, and it is also crucial for the host to respond and eradicate pathogens. In eukaryotes, regulation of message stability contributes significantly to the control of gene expression and plays a prominent role in the normal physiology of a cell as well as in its response to environmental and pathogenic stresses. Not surprisingly, emerging evidence indicates that there are significant interactions between the eukaryotic RNA turnover machinery and a wide variety of viruses. Interestingly, in many cases viruses have evolved mechanisms not only to evade eradication by these pathways, but also to manipulate them for enhanced viral replication and gene expression. Given our incomplete understanding of how many of these pathways are normally regulated, viruses should be powerful tools to help deconstruct the complex networks and events governing eukaryotic RNA stability. © 2010 John Wiley & Sons, Ltd.
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The virus–host interaction is akin to an evolutionary arms race; viruses continually evolve and fine-tune mechanisms to exploit the cellular machinery for their own replication, while the host counter-attacks using a multitude of strategies to detect and destroy foreign invaders. Many such 'molecular battles' are waged to control gene expression, as commandeering this machinery is crucial to the success of all viral infections. Given that the regulation of cellular gene expression is orchestrated in large measure by changes in messenger RNA (mRNA) stability, viruses must interface with the cellular RNA decay pathways to control the levels of cellular and viral RNAs. In addition, the cellular RNA destruction machinery likely represents a formidable obstacle to viral genomic and/or mRNA accumulation and integrity. Therefore, while viral RNAs can be targeted for turnover by the host machinery, in many cases viruses have evolved to circumvent and even subvert these pathways to facilitate their own replication. This review will highlight both these themes in relation to the variety of cellular regulators and effectors controlling the ultimate fate of RNA in an infected cell. We will focus on how viruses are influenced by the basal mRNA decay machinery, as well as pathways governing mRNA quality control. Although an extensive body of literature also exists for microRNA and small interfering RNA-mediated regulation of viral and cellular gene expression during infection, this topic is reviewed elsewhere in this journal and is thus not covered herein.

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DEADENYLATION

Cellular mRNAs are protected at their termini by a 5′ 7-methylguanosine cap and a 3′ non-templated poly(A) tail, which facilitate translation and restrict access of exonucleases. Within the nucleus, deadenylases trim poly(A) tails for mRNA export and participate in the destruction of non-coding or aberrant transcripts.[1] In the cytoplasm, poly(A) tail removal is the first and often the rate-limiting step of general mRNA turnover and is usually required both for subsequent decapping and degradation of the message body[2] (Figure 1). Several deadenylase complexes have been identified, including Ccr4-Caf1-Not1, PAN2-PAN3, and PARN.[3] While to date no viruses have been shown to directly interfere with specific deadenylases, several examples exist of viral RNAs evading the general deadenylation process.

Alphaviruses such as Sindbis virus (SINV) and Venezuelan equine encephalitis virus (VEEV) are positive-sense single-stranded RNA [(+)RNA] viruses whose genomic RNAs are both capped

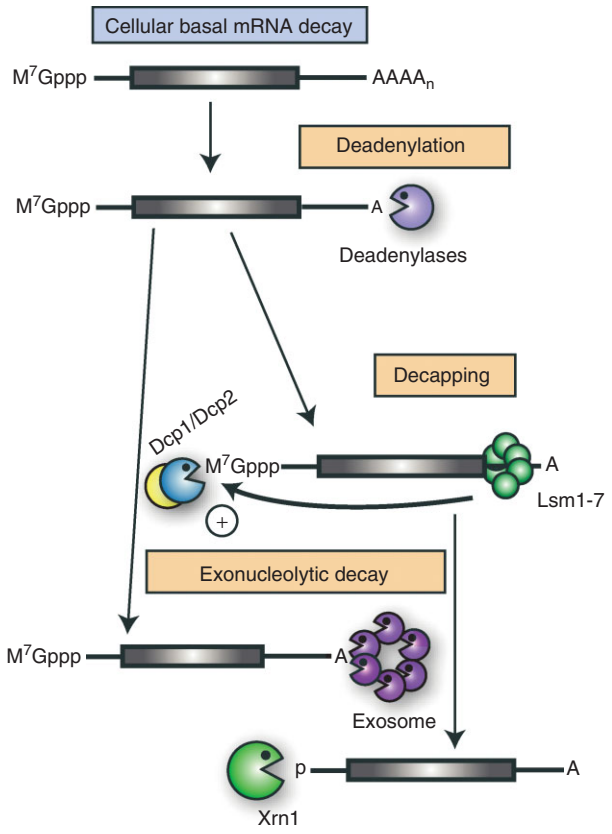

FIGURE 1 | Basal pathways of cellular mRNA decay. Degradation of normal cellular mRNA initiates with removal of the poly(A) tail by one of the cellular deadenylases. Subsequently, the Lsm1–7 protein complex binds the 3′ untranslated region (UTR) of the deadenylated messages and stimulates decapping by the Dcp2 enzyme and its activator protein Dcp1. The message body is subject to 3′→5′ exonucleolytic decay by the exosome or 5′→3′ decay by Xrn1.

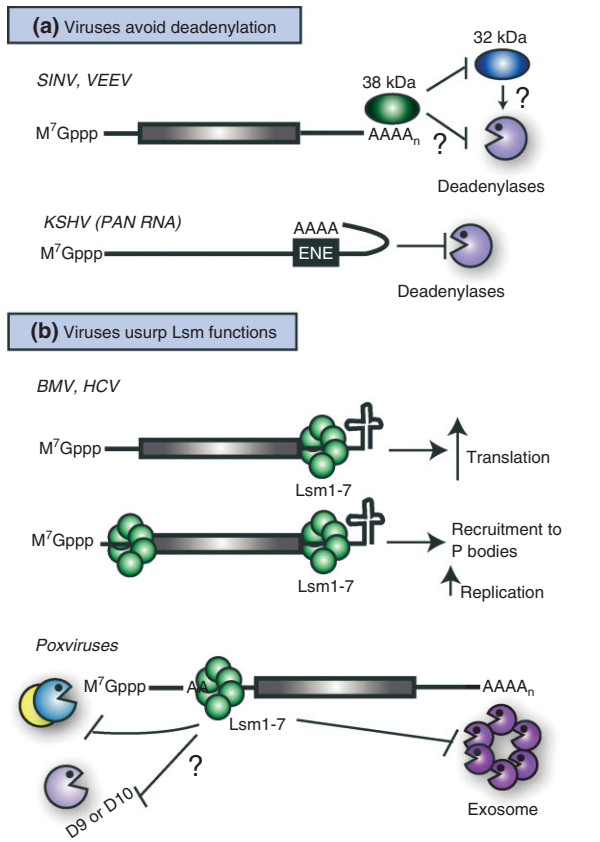

FIGURE 2 | Viral circumvention or utilization of cellular deadenylation and decapping pathways. (a) The RNAs of Sindbis virus (SINV) and Venezuelan equine encephalitis virus (VEEV) contains elements in the 3′ UTR that recruit a cellular 38-kDa protein and block deadenylation, presumably by preventing binding of a 32-kDa deadenylation-promoting factor. The PAN RNA of Kaposi's sarcoma-associated herpesvirus contains an element (ENE) that interacts in *cis* with the poly(A) tail thus preventing deadenylase access. (b) The RNA genomes of Brome mosaic virus (BMV) and hepatitis C virus (HCV) can be bound by Lsm proteins in their 3′ and/or 5′ UTRs. This enhances their translation, as well as facilitates replication, possibly through recruitment of the RNAs to P bodies. Poxviruses transcripts have polyadenine sequences within their 5′ UTRs, which bind Lsm1–7. Lsm binding prevents decapping (and presumably 5′→3′ decay), as well as 3′→5′ degradation of the messages.

and polyadenylated, resembling cellular mRNAs. Consequently, to avoid degradation, SINV and VEEV RNAs have evolved sequences that can stall deadenylation[4] (Figure 2(a)). A combination of conserved sequences in the 3′ untranslated region (UTR) of the viral genomes act in *cis* to repress deadenylation in mosquito cell extracts, presumably by mediating interaction with a protective cellular factor.[4] A 38-kDa mosquito protein binds both the SINV and the VEEV 3′ UTRs and can be specifically titrated away in the presence of excess viral UTR sequences, leading to decreased viral RNA stability. Interestingly, removal of the 38-kDa protein from SINV RNA leads to increased binding of a 32-kDa mosquito protein. This suggests a mechanism whereby the viral UTRs may be stabilized by displacing a deadenylation-promoting factor via preferential binding to a protective factor. The identity of these two cellular proteins and their conservation across species is currently unknown. However, in the future their characterization will likely provide insight both into how both cellular mRNA deadenylation and viral RNA stabilization are orchestrated.

A second mechanism of protection from deadenylation is used by a highly abundant non-coding nuclear RNA of Kaposi's sarcoma-associated herpesvirus (KSHV), termed PAN (Figure 2(a)). PAN contains a 79-nt element (ENE) near its 3′ end that acts post-transcriptionally to stabilize the RNA and promote its nuclear retention.[5] The ENE forms a stem-loop structure containing a U-rich loop. This loop makes intramolecular contacts with the PAN

poly(A) tail and blocks deadenylation, presumably in part by preventing access of deadenylases to the RNA.[6] However, a simple U-rich stretch is not sufficient to recapitulate the effects of PAN-ENE,[6] and mutations in the stem region of PAN-ENE can also abolish its function.[7] These observations suggest that the entire structure is required for efficient interaction and potentially for the recruitment of additional protective factors. As the function of PAN remains unknown, at present the importance of the PAN-ENE in KSHV infection cannot be assessed. The fact that the decay kinetics of PAN are different from those of cytoplasmic polyadenylated RNAs has led to the speculation that PAN RNA degradation, particularly at the stage of deadenylation, may be carried out by a dedicated nuclear pathway that normally disposes of incorrectly processed RNAs.[6] Thus, the study of PAN RNA protection from nuclear degradation may provide information as to how polyadenylation and deadenylation are carried out in the nucleus of mammalian cells and how they contribute to nuclear RNA quality control.

THE Lsm COMPLEX

As deadenylation is completed, a ring-shaped heptameric complex of Sm-like proteins (Lsm1–7) binds to the 3′ end of the message and recruits the cellular decapping enzymes Dcp1 and Dcp2, which promote cap hydrolysis[8–11] (Figure 1). The Lsm complex is highly conserved in eukaryotes, and in yeast it has been shown to associate with several additional components of the RNA decay machinery, including decapping activators and the 5′→3′ exonuclease Xrn1.[10–14] While the role of the Lsm proteins in eukaryotes is closely linked to mRNA degradation, several viruses use this complex instead to facilitate viral replication and, surprisingly, to enhance viral RNA stability.

The Lsm complex has been shown to participate in both viral replication and translation of Brome mosaic virus (BMV)[15–17] and hepatitis C virus (HCV)[18] (Figure 2(b)). Like those of all other (+)RNA viruses, the genomes of BMV and HCV are used first as mRNAs for protein production, and subsequently as templates for replication. These two processes are considered mutually exclusive and often require different *cis*-acting sequences. The genome of BMV consists of three RNA segments that are 5′ capped, but terminate in a 3′ tRNA-like structure rather than a poly(A) tail. Although plants are its natural host, BMV can also replicate in yeast, which has greatly facilitated genetic dissection of host factors involved in its replication cycle. A mutagenesis screen in yeast identified Lsm1p as a host factor required for BMV RNA replication.[15] Subsequent studies showed that Lsm1p, together with other components of the Lsm complex, is also required for efficient BMV genomic RNA translation elongation, but not initiation.[17] While Lsm-stimulated BMV translation requires multiple regions of the viral RNAs, the effect of Lsm on BMV RNA replication is specifically dependent on 3′ UTR elements.[16,17] Thus, partially overlapping signals appear to participate in the seemingly opposing activities of translational enhancement and redirection of RNAs from the translational pool into replication complexes. Notably, Lsm proteins do not regulate the translation of the BMV coat protein from a fourth subgenomic RNA segment, RNA4, which is generated during BMV replication, suggesting that the effects of Lsm proteins on BMV RNA translation and replication are tightly linked.[17]

As mentioned before, the Lsm complex is similarly important for the HCV lifecycle, suggesting that the observations on the role of Lsm in BMV replication and translation may generalize to many (+)RNA viruses. Indeed, siRNA-mediated knockdown of Lsm1 and its co-activators PatL1 and Rck/p54 decreases HCV intracellular RNA levels, translation, and viral particle production.[18] Using gel shift assays, the Lsm1–7 ring was shown to interact with select HCV 5′ and 3′ UTR sequences necessary for translation and replication, suggesting that it may play a direct role in modulating these processes during infection.

It has been suggested that the Lsm proteins may direct BMV RNAs to replication complexes by recruiting them to P bodies, which are sites of RNA storage and/or decay[19] (Figure 2(b)). Because P bodies are thought to hold mRNAs in a translationally repressed state,[20,21] in principle they could be ideal sites for viral genome replication.[19] Surprisingly, when the BMV and HCV 3′ tRNA-like structure are replaced with a poly(A) tail, the Lsm complex is no longer required for viral RNA accumulation and translation.[16–18] One hypothesis is that these viruses co-opt Lsm to compensate for the RNA stabilizing functions normally provided by polyadenylation and its associated poly(A) binding protein (PABP). However, association with PABP should not result in RNA recruitment to P bodies, as PABP is not a component of P bodies.[22] In addition, the mRNAs associated with P bodies are generally believed to be deadenylated.[23] Therefore, it is not clear that PABP and Lsm association could substitute for each other in this system. Further studies are therefore needed to delineate how and why the Lsm complex is required for viral RNA replication only in the absence of polyadenylation.

Cellular Lsm proteins may also play a role in the stability of mRNAs of poxviruses, which are large DNA viruses that replicate in the cytoplasm (Figure 2(b)). Transcripts from orthopoxviridae, particularly those expressed later in infection, often contain non-templated adenosine stretches of 5–40 nucleotides within their 5′ UTRs, thought to be generated by polymerase stuttering.[24] 5′ adenosine tracts of at least 10 nt were shown to promote mRNA stabilization in cells and in cytoplasmic extracts by inhibiting both decapping and 3′→5′ exonucleolytic decay of the RNA.[25] Although these adenosine stretches bind PABP, titrating away PABP with excess poly(A) RNA does not affect the stability of the 5′ adenosine tract-containing transcripts. Their stabilization instead correlates with direct binding of the Lsm1–7 complex.[25] Poxviruses encode two decapping proteins, D9 and D10, which contribute to the global cellular mRNA turnover that occurs during poxvirus infection. However, D9/D10 can also target viral transcripts and may restrict expression of early viral genes.[26–28] Thus, Lsm proteins may participate in the protection of late viral messages from the viral decapping proteins, although it has yet to be directly proven that this complex protects viral messages during infection or that it blocks D9/D10 decapping activity.[25]

It is notable that the virus-associated roles of the Lsm complex in mRNA stabilization, enhanced translation, and replication are at odds with its established cellular function in activating mRNA decapping to facilitate message degradation. While it is formally possible that these viruses are manipulating the Lsm complex to induce novel activities, a more likely explanation is that the Lsm proteins have multifaceted roles in cellular gene expression, which are being revealed and exploited by viruses.

5′→3′ mRNA DECAY

Decapping exposes the mRNA 5′ end, triggering rapid exonucleolytic destruction of the message body predominantly via the 5′→3′ nuclease Xrn1 (Figure 1). Viruses lacking a 5′ cap might therefore be particularly susceptible to Xrn1-mediated degradation. Indeed, Xrn1 was initially discovered in the yeast *Saccharomyces cerevisiae* for its 'Superkiller' (Ski) phenotype: loss of Xrn1 resulted in cell death as a consequence of increased levels of the toxin-encoding uncapped M satellite double-stranded RNA (dsRNA) of the L-A virus.[29] In addition, both in yeast[30] and in the plant *Nicotiana benthamiana*,[31] Xrn1 and its homolog Xrn4 prevent the genomic RNA of Tomato bushy stunt virus from accumulating (Figure 3(a)). These exonucleases also reduce the levels of viral RNA intermediates necessary for recombination, a process that some RNA viruses exploit to repair their genomes and increase diversity.[32]

How do RNA viruses avoid Xrn1-mediated degradation? Some protect their RNAs by encoding *cis* elements that can physically block Xrn1. The yeast 20S RNA narnavirus, for example, has a highly structured G-rich motif in its 5′ region that can block Xrn1 activity, thereby likely enhancing the 20S virus persistence in yeast laboratory strains[33] (Figure 3(b)). In contrast, poliovirus (PV) recruits a ribonucleoprotein (RNP) complex including poly(rC) binding proteins to a 5′ cloverleaf RNA structure[34] (Figure 3(b)). Mutations in this structure and consequent loss of the RNP adversely affect PV RNA stability, possibly as a consequence of increasing susceptibility to Xrn1-mediated degradation.[35] This strategy is probably common to many other members of the *Picornaviridae* family, as the 5′ cloverleaf structure is highly conserved.[36]

Interestingly, some viruses instead exploit the activity of Xrn1 (Figure 3(c)). Flaviviruses such as West Nile and Dengue viruses may appropriate Xrn1 to produce a 3′ subgenomic RNA (termed sfRNA) that plays an essential role in viral pathogenicity in both cell culture and mice.[37] These viruses have a highly structured sequence (stem loop II or SLII) within the 3′ UTR of their genome that is necessary for the production of the sfRNA. Depletion of Xrn1 prevents production of sfRNA, suggesting that sfRNA is a product of Xrn1-mediated degradation of the viral genomic RNA upstream of SLII. In turn, this indicates that the SLII element can stall Xrn1 activity. Because no other Xrn1-blocking element has been described in mammalian cells, the SLII element will likely prove a valuable tool to study Xrn1-mediated degradation both in the context of viral infections and in uninfected cells.

3′→5′ mRNA DECAY

The exosome, a multisubunit protein complex with 3′→5′ exonucleolytic activity, is the major driver of RNA degradation from the 3′ end (Figure 1). In the cytoplasm, this complex participates in mRNA turnover after deadenylation, whereas in the nucleus it plays an essential role in RNA processing and quality control.[38] Little is currently known about the interaction of the exosome with viruses. However, the zinc-finger antiviral protein (ZAP), an important mediator of cellular response to retroviruses,[39] alphaviruses,[40] and filoviruses,[41] has been shown to bind the hRrp46 component of the exosome and to recruit the complex to viral mRNAs to promote their degradation.[42] Depletion of the exosome using siRNAs directed against the hRrp41 and hRrp46

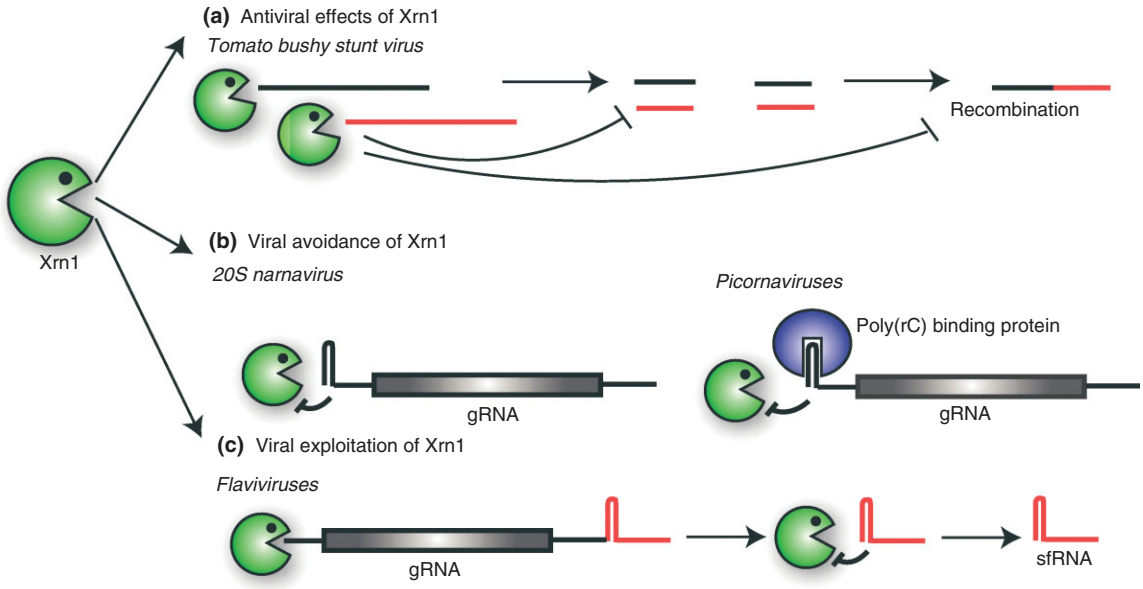

FIGURE 3 | Viral interactions with Xrn1. (a) During infection with Tomato bushy stunt virus, Xrn1 can prevent the accumulation of viral RNA and of viral RNA intermediates that serve as substrates for recombination. (b) Some viruses prevent Xrn1 degradation of their uncapped genomic RNAs (gRNA) by having highly structured sequences in the 5′ UTR, as in the case of 20S narnavirus, or by recruiting protein complexes to this region, as in the case of Picornaviruses. (c) Flaviviral RNAs contain Xrn1 blocking sequences in their 3′ UTR. Xrn1-mediated degradation of the RNAs up to the blocking sequence results in the generation of a functional subgenomic RNA, sfRNA.

subunits reduces turnover of reporter RNAs by ZAP, suggesting that exosome activity is an integral component of ZAP function. ZAP binds viral RNAs in a sequence-specific manner, perhaps explaining why this protein is not a broad-spectrum viral inhibitor.[43] However, the absence of significant homology between the various viral sequences bound by ZAP implicates RNA structure as the primary determinant for recognition. An important next step will be to directly test whether exosome depletion rescues viral replication defects in ZAP-expressing cells.

These findings suggest a role for the exosome in controlling viral infection. In contrast, no evidence currently exists for viruses directly co-opting this complex. Nonetheless, given the widespread roles for the exosome in RNA quality control and turnover, viruses presumably activate this complex at least indirectly when triggering turnover of cellular mRNAs. For example, infection by select herpes and coronaviruses results in a global destruction of host messages.[44–48] The exosome, as well as other RNA turnover pathways, may contribute to degradation of full-length or partially degraded mRNAs in these cases.

mRNA QUALITY CONTROL

Cells are equipped with specific mechanisms for the recognition and disposal of defective mRNAs generated by errors introduced during transcription and RNA processing or as a result of mutations in the DNA sequence. The best characterized of these pathways is nonsense-mediated decay (NMD), which targets mRNAs harboring a premature stop codon (PTC) (Figure 4). The core NMD effectors are the Upf proteins, which associate with prematurely terminating mRNAs and trigger their rapid destruction. Current models for NMD activation in human cells suggest that Upf1 is recruited to these aberrant mRNAs during the initial round of translation via interactions with the eRF1 and eRF3 translation termination factors, whereupon other effectors of NMD (including Upf2, Upf3, and Smg proteins) join the complex.[49] The aberrant mRNA is then cleaved endonucleolytically near the PTC by the NMD factor Smg6, and the message body is degraded by cellular exonucleases[50,51] (Figure 4). Two features can mark mRNAs for destruction via the NMD pathway. In human cells, one important feature is the presence of exon–exon junctions downstream of the termination codon, as determined by the retention of exon-junction complexes (EJCs) at these sites. Normally EJCs, which are deposited during splicing, are displaced from the mRNA by translating ribosomes.[52–54] However, NMD can also occur in the absence of splicing, suggesting that downstream EJCs are not always required for activation of NMD.[49] Indeed, a second determinant is the length of the 3′

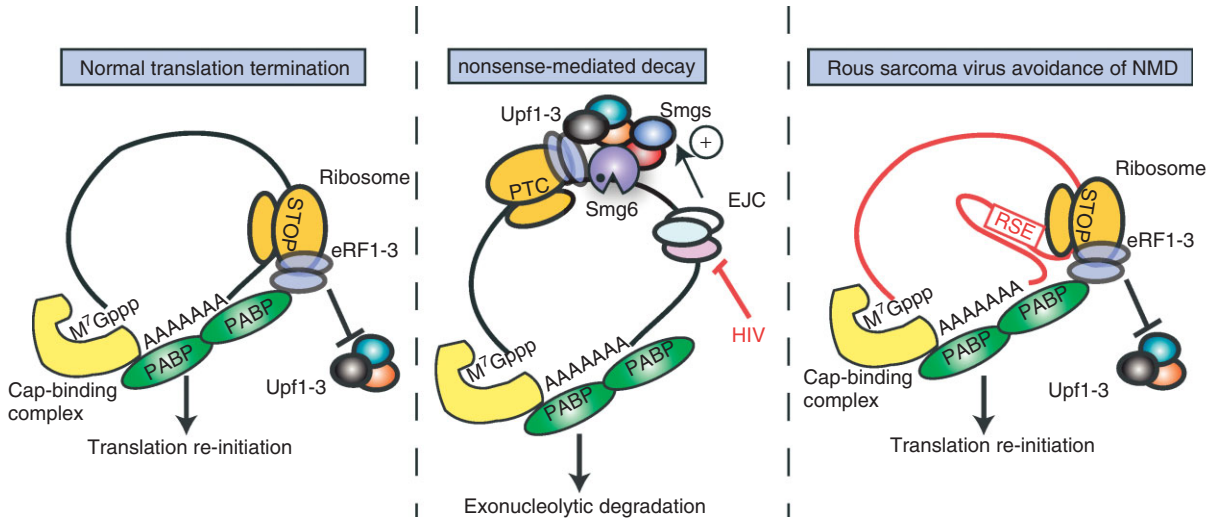

FIGURE 4 | Nonsense-mediated mRNA decay and mechanisms of viral avoidance. (a) When the translation machinery reaches the stop codon of a normal mRNA, the eukaryotic release factors eRF1 and eRF3 are recruited to the ribosome, in part via interactions with PABP. This results in efficient translation termination and re-initiation. (b) When mRNAs containing a premature stop codon (PTC) are translated, the presence of EJCs downstream of the PTC, coupled with inefficient interactions between PABP and the termination factors, triggers nonsense-mediated decay (NMD). NMD is induced by recruitment of Upf1–3 and the Smg proteins; cleavage by the endonuclease Smg6 triggers degradation of the mRNA fragments. Viruses such as HIV-1 regulate the alterative splicing of their genomic RNAs to avoid the presence of EJCs downstream of stop codons. (c) The unspliced RNA of Rous sarcoma virus has a very long 3′ UTR, which is stabilized by the RSE element that directly inhibits NMD. This element may artificially 'shorten' the 3′ UTR by making contacts with sequences close to the poly(A) tail.

UTR, which is monitored by the proximity of the termination codon to the poly(A) tail. Messages bearing PTCs possess longer 3′ UTRs than their wild-type counterparts and this appears to decrease the efficiency of translation termination and facilitate Upf1 recruitment, even in the absence of downstream EJCs.[55–57]

NMD represents a potentially formidable obstacle for viruses, many of which encode polycistronic transcripts that would presumably be viewed by the cellular NMD machinery as having aberrantly long 3′ UTRs. Additionally, viruses frequently use alternative splicing to maximize coding potential, possibly creating spliced variants with EJC components remaining downstream of the stop codon used by the 5′ ORF. One way viruses may escape NMD is by tightly regulating their splicing pattern, as is the case with human immunodeficiency virus 1 (HIV-1).[58] The sole HIV-1 transcript is alternatively spliced to generate more than 30 mRNAs. The genes are always spliced out sequentially in a 5′→3′ order, so as to avoid generating transcripts where splicing of 3′ genes leads to deposition of EJCs downstream of the termination codons of the 5′ genes. However, a consequence of this strategy is that the 5′-most genes are generally translated from completely unspliced mRNAs, which have very long UTRs between the stop codon of the first gene and the poly(A) tail. Thus, some viruses specifically avoid NMD by encoding protective elements within their long 3′ UTRs. This is best illustrated by the avian retrovirus Rous sarcoma virus (RSV), which contains a sequence element (RSE) downstream of the Gag gene stop codon that prevents the unspliced mRNA from being targeted for degradation.[59] Deletion of this sequence causes a dramatic shortening of the half-life of unspliced RSV mRNA in a translation- and Upf1-dependent manner, whereas insertion of the RSE protects PTC-containing NMD substrates from degradation. Analyses of the RSE structure using SHAPE chemistry and partial RNase digestion indicate the presence of an AU-rich stretch and stem-loop elements that are conserved amongst 20 different avian retroviruses.[60] While the mechanisms by which the RSE blocks NMD remain unknown, an intriguing preliminary observation suggests that it may form an RNA–RNA interaction with a region just upstream of the RSV poly(A) site.[60] Given the correlation between the proximity of the stop codon and poly(A) tail for NMD activation, such an interaction could facilitate translation termination by bringing the poly(A) tail closer to the Gag stop codon, thereby decreasing Upf1 recruitment (Figure 4). Alternatively, the RSE may bind proteins that influence the stability of the mRNA.

It will be of interest to examine whether similar, perhaps structurally related elements exist within

other viral or cellular mRNAs with unusually long 3′ UTRs, as this may shed light on how this important pathway is regulated normally in cells and in response to infection. For example, both coronavirus and SINV have long stretches of coding sequence at their 3′ end that are not translated from the genomic mRNA, but instead are expressed from subgenomic transcripts.[61,62] During the translation of genes at the 5′ end of these viral genomes such sequences would appear as very long 3′ UTRs, which might be predicted to trigger NMD even in the absence of nuclear splicing and EJC deposition. Thus, a protective mechanism may be required to actively stabilize the RNA genomes in such cases. A similar situation could arise during infection with some DNA viruses such as those of the gamma-herpesvirus subfamily, which transcribe a number of potentially polycistronic messages.[63]

Additional, NMD-independent roles in viral infection have been proposed for Upf1. For example, Upf1 associates with HIV-1 RNA, and its presence stabilizes the viral genome and facilitates translation of the Gag precursor.[64] These functions of Upf1 are genetically separable from its role in NMD, as they are observed even with mutants of the Upf1 RNA helicase domain that have dominant-negative effects on NMD.

An intriguing idea is that cells may specifically transform viral mRNAs into targets of quality control pathways as an antiviral measure. Evidence for this comes from studies with the pokeweed antiviral protein (PAP), a ribosome-inactivating factor isolated from the pokeweed plant *Phytolacca americana* that functions via RNA depurination.[65–67] This protein has a broad-spectrum antiviral activity against both plant and animal viruses, including HIV,[68] and has generated interest as a potential antiviral treatment.[69] Although initially antiviral activity was ascribed to the ribosome-inactivating function of PAP, it was subsequently found that some of the PAP mutants that are unable to depurinate ribosomal RNA (rRNA) can still selectively inhibit viral replication.[70] PAP was subsequently shown to depurinate capped mRNAs, and the aforementioned mutants were shown to retain the ability to depurinate mRNAs.[66] Recent studies in yeast using BMV have shown that BMV RNAs become depurinated upon PAP expression, leading to ribosome stalling and enhanced RNA turnover[71] (Figure 5). Interestingly, PAP-induced turnover of BMV RNA did not occur in yeast defective in the Dom34p endonuclease, a key component of a quality control pathway termed No-go decay (NGD). NGD is a surveillance mechanism that targets mRNAs with translation-elongation stalls, for example as a consequence of rare codons or strong secondary structures. Following endonucleolytic cleavage by

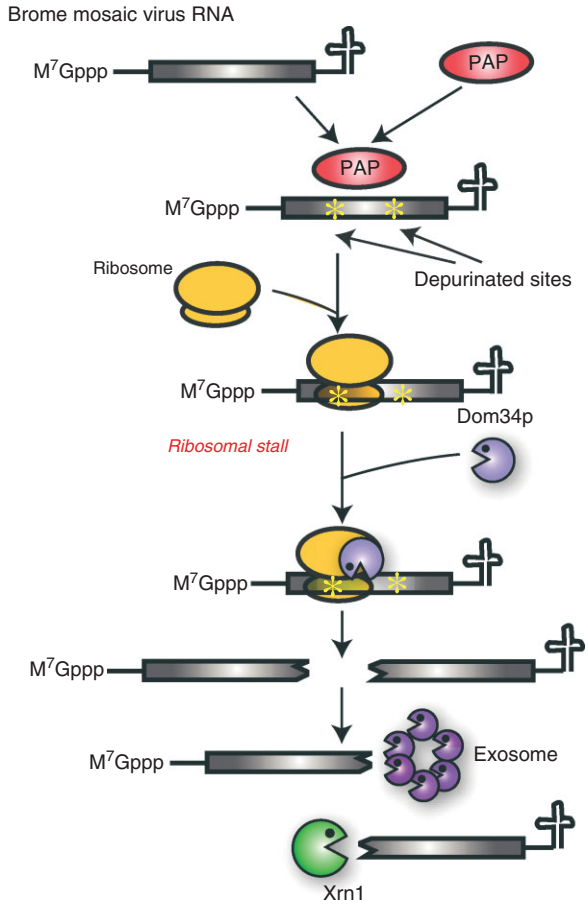

FIGURE 5 | No-go decay (NGD) as an antiviral pathway. In yeast ectopically expressing the plant protein PAP, the RNAs of Brome mosaic virus are depurinated. This causes translating ribosomes to stall, thereby triggering NGD via recruitment of the endonuclease Dom34p to the translation complex. After Dom34p cleaves the RNA close to the site of stalling, the resulting fragments become targets for exonucleases such as Xrn1 and the exosome.

Dom34p, the resulting RNA fragments are degraded from the 5′ end by the exonuclease Xrn1 and from the 3′ end by the exosome[72] (Figure 5). As expected for NGD, yeast mutants defective in Xrn1 or exosome activity accumulate cleaved BMV RNA intermediates.

While NGD has been demonstrated in yeast, an analogous surveillance pathway in mammalian cells has yet to be identified. Thus, it will be especially interesting to determine whether PAP antiviral activity against mammalian viruses similarly involves depurination followed by translation-dependent RNA turnover, as this would provide compelling evidence for NGD in humans. Along these lines, a recent study found a reduction in the RNA levels of human T-cell leukemia virus type I (HTLV-I), as well as viral RNA depurination, in mammalian cells

that ectopically express PAP.[73] However, unlike the situation in yeast with BMV, HTLV-I RNAs were not destabilized, arguing against an NGD-like mechanism of suppression. It is noteworthy that whereas a number of N-glycosidases that depurinate rRNA have been described in plants,[74] no such protein has been reported in animal cells. In addition, it will be important to elucidate which features differentiate PAP-susceptible versus non-susceptible RNAs in light of potential antiviral uses for PAP.

RNase L

In eukaryotic cells, endonucleases are critical components of multiple cytoplasmic quality control pathways but may not be major participants in basal mRNA decay. However, an interferon-inducible riboendonuclease, RNase L, has important roles in the innate immune response to pathogens. RNase L is activated in the presence of dsRNA, a non-self species often present in virally infected cells (Figure 6) (reviewed in Ref 75). dsRNAs stimulate the activity of 2′,5′-oligoadenylate synthetase (OAS), which produces the allosteric activator for RNase L, 2′-5′-oligoadenylate (2–5A). Activated RNase L cleaves at UU and UA dinucleotides and contributes to the host defense by destroying not only viral RNAs, but also cellular mRNAs and rRNAs that are needed for viral replication, and by stimulating apoptosis. In addition, cleavage by RNase L liberates small dsRNA species that activate the pattern recognition receptors RIG-I and Mda-5, leading to IFN-β production and amplification of the antiviral interferon response.[76]

dsRNA is most often found upon infection with RNA viruses that produce dsRNA intermediates during genome replication, although dsDNA viruses can also produce dsRNA due to annealing of complementary RNAs transcribed from opposite strands of the genome. RNase L-deficient cells and mice thus exhibit increased susceptibility to infection by several viruses (reviewed in Ref 75) including encephalomyocarditis virus,[77] SINV,[78] and Coxsackievirus B4.[79] It has also been proposed that a human reduction-of-function mutation in RNase L renders individuals more susceptible to xenotropic murine leukemia-related virus, a retrovirus that has been linked to prostate cancer and chronic fatigue syndrome.[80,81] A recent report has shown that in the absence of RNase L, mice infected with a neurotropic demyelinating strain of mouse hepatitis virus (MHV) succumb to CNS encephalomyelitis.[82] Notably, the infected RNase L-/- mice did not exhibit increased neuronal infection, interferon induction, or viral titers; mortality upon infection correlated instead with advanced demyelination and CNS tissue damage. Thus, RNase L somehow prevents extended and sustained CNS tropism of MHV. The dramatic effects of such subtle tropism alterations highlight both the diversity of RNase L functions as well as its complex interactions with infecting viruses.

Not surprisingly, many viruses have evolved mechanisms to blunt the activation of RNase L, and such inactivation occurs at a variety of different steps in the RNase L pathway (Figure 6). The NS1 protein of influenza A virus, for example, acts at a very early step by coating dsRNA and thereby preventing these species from activating OAS.[83,84] Infection with vaccinia virus (VV) or HIV-1, on the other hand,

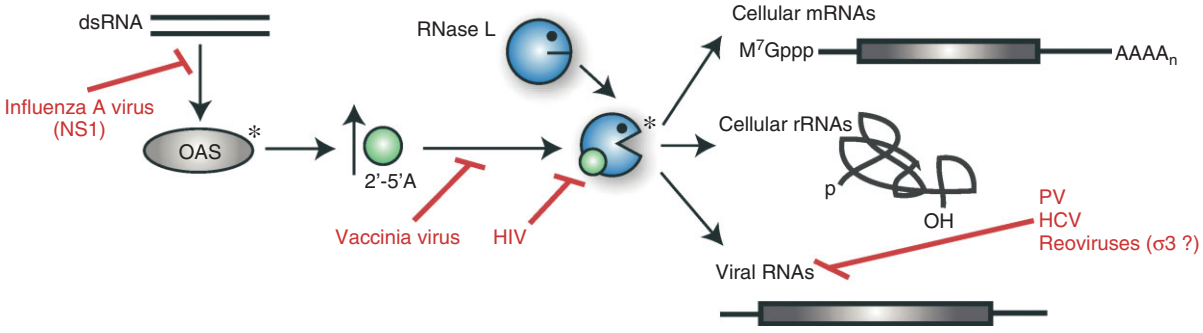

FIGURE 6 | Viruses interfere with several stages of the RNase L pathway. RNase L is indirectly activated by the presence of double-stranded RNA (dsRNA) species in the cytoplasm of infected cells. dsRNA activates 2′,5′-oligoadenylate synthetase (OAS), which produces 2′-5′-oligoadenylate (2–5A), an allosteric activator for RNase L (asterisks indicate active enzymes). The NS1 protein of influenza virus A prevents OAS activation, whereas vaccinia virus and HIV-1 block activation of RNase L by 2–5A, the latter by decreasing the affinity of RNase L for its activator. Once activated, RNase L can cleave viral RNAs and also cellular mRNAs and rRNAs. Several viruses specifically protect their RNAs from degradation, including poliovirus (PV), hepatitis C virus (HCV), and possibly reoviruses, using their σ 3 protein. Reoviruses also exploit RNase L-mediated destruction of cellular messages to decrease competition for gene expression machinery.

potently induces 2–5A production, but subsequent RNase L activation is blocked.[75,85,86] In the case of HIV-1, it appears that this is accomplished by somehow decreasing the affinity of RNase L for 2–5A (reviewed in Ref 85).

Some viruses, like HCV and PV, do not block RNase L activation, but their RNAs still escape degradation by this endonuclease. It has been shown that select genotypes of HCV avoid RNase L cleavage by suppressing the frequency of UU and UA sequences in their genome, perhaps as a consequence of selective evolutionary pressure.[87] HCV genotypes in the human population that are less sensitive to RNase L also show increased interferon resistance.[87] Conversely, the PV genome is very UU/UA rich, but is still surprisingly resistant to RNase L cleavage because of a structural element present within the PV RNA2 ORF.[88] This element functions as a competitive inhibitor of RNase L thereby directly blocking its enzymatic activity.[89] The role of RNase L inhibition during PV infection remains unclear, however, as PV carrying mutations that prevent the formation of the inhibitory structural element replicate to wild-type levels in cell culture, and do not exhibit increased sensitivity to interferon.[88]

Although the above examples highlight RNase L as an antiviral protein, in some cases it may facilitate viral replication and/or dissemination. For example, PV forms larger plaques if cultured in cells overexpressing wild-type RNase L, and smaller plaques in cells overexpressing a dominant-negative form of the protein.[88] It has been hypothesized that PV selectively inhibits RNase L early in infection, but then uses RNase L-induced apoptosis to enhance viral release and spread. Mammalian reoviruses may subvert the host shutoff activity of RNase L to dispose of cellular mRNAs and clear the gene expression machinery for viral use.[90] Replication of both the Jones and Dearing reovirus strains was reduced by ~1 log in RNase L knockout relative to wild-type mouse embryo fibroblasts, supporting the idea that the virus partially co-opts this pathway to facilitate infection.[90] Interestingly, minimal degradation of rRNAs was observed, suggesting that RNase L may not act indiscriminately as originally thought, but rather target specific sequences.[91–93] The mechanism of viral escape from these pathways remains unknown, although the reovirus $\sigma 3$ protein has been proposed to coat viral dsRNAs, thereby selectively inhibiting RNase L activation in cytoplasmic locales where viral RNA replication occurs, i.e., 'replication factories'.[94] However, it should be noted that the results mentioned above contrast with earlier findings implicating RNase L in restricting reovirus replication, which may be due to the use of different cell types in these studies.[95,96]

PERSPECTIVES

In addition to the examples highlighted herein, in many other cases viral infection presumably leads to robust engagement of cellular mRNA turnover machinery. For example, several viruses remove the 7-methylguanosine caps from cellular mRNAs, prevent appropriate cellular mRNA splicing, 3′ processing, or polyadenylation, or directly cleave cellular messages.[27,46,97–100] Presumably these events lead to destruction of the impaired cellular transcripts through both basal and quality control machinery operating in the nucleus and in the cytoplasm. Such elevated global RNA degradation may overload the RNA turnover machinery, potentially indirectly stabilizing viral transcripts. Aside from affecting global RNA levels, viruses can also selectively alter the stability of specific messages through the induction of cellular signaling pathways. For example, the activation of the MK2/p38 pathway by the kaposin B protein of KSHV leads to stabilization of cytokine mRNAs bearing AU-rich elements in their 3′ UTRs.[101] In these cases, viral manipulation of select transcript stability likely has important consequences for overall pathogenesis.

Our understanding of the multitude of ways in which viruses either commandeer or are controlled by cellular mRNA turnover pathways is constantly evolving, and far from complete. Moreover, many of the pathways governing cellular mRNA decay remain incompletely understood even in uninfected cells, and new components and mechanisms are constantly described. Thus, as in many other fields of biology, viruses will continue to be invaluable tools for revealing key modulators of mRNA stability and destruction both in normal and pathogenic states.

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